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(57) Abstract

The present invention comprises spumavirus isolated from humans. More specifically, the spumavirus of the present invention was isolated from humans who had exposure to nonhuman primates. Importantly, the spumavirus of the present invention or antibodies to the spumavirus can be used to detect the presence of spumavirus or antibodies in body fluids, for pathogenicity studies of related viruses, and as a vector for gene therapies. The spumavirus of the invention can also be used for treatment of conditions in humans due to the presence of rapidly dividing cells and for recombinant live virus vaccination.

NEW SPUMAVIRUS ISOLATED FROM HUMANS

Technical Field

The present invention relates to a novel retrovirus, a spumavirus, that has been isolated from humans. More particularly, the novel spumavirus may be used as a vector for gene therapy. The novel spumavirus may also be used as a recombinant live virus vaccine.

Background of the Invention

Spumavirus, also known as foamy virus for the characteristics of vacuolization the virus induces in cell culture, belongs to a distinct group of retroviruses. The simian foamy viruses (SFVs) include isolates from Old World and New World monkeys and are classified into 10 different serotypes based on serological cross-reactivities. Virus appears to persist in the host for a long period of time in a latent form and can exist in the presence of neutralizing antibody.

Currently the most studied retrovirus, Human Immunodeficiency Virus, is believed to be derived from nonhuman primate transmission into humans at some past time. Concerns about the risk of transmission of retroviruses from non-human primates to humans working in research laboratories were heightened in the early 1990's when two persons developed antibodies to SIV (Simian Immunodeficiency Virus) following work-related exposures, one of whom had clear evidence of persistent viral infection. (See CDC. Anonymous survey for simian immunodeficiency virus (SIV) seropositivity in SIV laboratory researchers -- United States, 1992. MMWR Morb Mort Wkly Rep 1992; 41: 814-5; Khabbaz R.F., et al. Brief report: infection of a laboratory worker with simian

immunodeficiency virus. New Eng J Med. 1994; 330: 172-7; Khabbaz RF, et al. Simian immunodeficiency virus needlestick accident in a laboratory worker. Lancet 1992; 340: 271-3; and CDC. Guideline to prevent simian immunodeficiency virus infection in laboratory workers and animal handlers. MMWR 1988; 37:693-704.) In addition to SIV, nonhuman primate species used in biomedical research are commonly infected with SFV (simian foamy virus), STLV (simian t-cell lymphotrophic virus), and/or type D retroviruses. All of these retroviruses cause lifelong infections in nonhuman primates, and some are known to be transmissible through sexual contact, blood, or breast feeding. Natural SFV infections in non-human primates have not been definitively associated with disease. In non-human primates, infection with the other retroviruses may result in a clinical spectrum ranging from asymptomatic infection to life threatening immunodeficiency syndromes or lymphoproliferative disorders. The transmission routes of SFVs among non-human primates remain undefined, but the prevalence of seroreactivity is high among captive adult non-human primates.

Studies of the prevalence of spumavirus infection of humans are limited and the findings are not definitive. Though there is some evidence of human infection with SFV (antibodies and positive PCR results), such occurrence has been reported in only two persons, both of whom had occupational risks for infection. Associated disease was not reported in either. (See Schweizer M., et al. Absence of foamy virus DNA in Graves' disease. AIDS Res & Human Retrov 1994; 10: 601-5; Neumann-Haefelin D, et al., Foamy viruses. Intervirology 1993; 35: 196-207; and Schweizer M, et al., Markers of foamy virus infections in monkeys, apes, and accidentally infected humans: appropriate testing fails to confirm suspected foamy virus prevalence in humans. AIDS Res & Human Retrov 1995; 11: 161-70.) There have been no published reports that virus was ever isolated from these infected individuals.

Other inconclusive evidence was seen in early studies which described a relatively high rate of seroreactivity to antibodies to spumaviruses among human populations not known to be exposed to non-human primates. In some instances seroreactivity was suggestively linked to human disease, including disorders of the central nervous system, thyroid disease, and Chronic Fatigue Syndrome. In most instances these studies

lacked definitive evidence of human infection and were not subsequently confirmed. (See Heneine W, et al., Absence of evidence for human spumaretrovirus sequences in patients with Graves' disease [letter]. J Acq Immune Defic Synd & Human Retrov. 1995; 9: 99-101; Simonsen L, et al.,. Absence of evidence for infection with the human spumaretrovirus in an outbreak of Meniere-like vertiginous illness in Wyoming, USA [letter]. Acta Oto-Laryngologica 1994; 114: 223-4; and Heneine W., et al., Lack of evidence for infection with known human and animal retroviruses in patients with chronic fatigue syndrome. Clin Infect Dis 1994; 18: S121-5).

To the knowledge of the inventors, there has not been a documented, definitive isolation of a spumavirus, such as the one of the present invention, from humans. Previous reports of human spumavirus isolates are now widely regarded as laboratory contaminants.

Recent publications indicate that earlier serological tests showing human spumavirus antibodies in the human population were incorrect. Immunological investigation of a previously reported human spumavirus revealed that it shared common antigens in complement fixation, immunofluorescence and neutralization assays with the chimpanzee foamy virus, SFV-6. Furthermore, failure to detect serological evidence of HFV infection in people from a wide geographical area suggested that this virus isolate was a variant of SFV-6, particularly since sera from chimpanzees naturally infected with SFV-6 neutralized both viruses. In a survey for prevalence of human foamy virus in more than 5000 human sera, collected from geographically diverse populations, none of the serum samples were confirmed as positive. Taken together with sequence analysis endorsing the phylogenetic closeness of the purported human spumavirus to SFV-6/7, these data strongly suggest that human foamy virus is not naturally found in the human population. (See Ali, M. et al., "No Evidence of Antibody to Human Foamy Virus in Widespread Human Populations," AIDS Research and Human Retroviruses, Vol. 12, No. 15, 1996.)

Recent concern that xenotransplantation, the use of living tissues from nonhuman species in humans for medical purposes, may introduce new infections into the human community has increased the importance of defining the ability of simian retroviruses to infect and/or cause disease in humans (See Chapman LE, et al. Xenotransplantation and xenogeneic infections. New Engl J Med 1995; 333: 1498- 1501; DHHS. Docket No.

96M-0311. Draft Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation. Federal Register Vol.61, No. 185. September 23, 1996.). The primary animal species considered as donors for xenografts are baboons and pigs. Thus, what is needed are compositions and methods for detecting viruses that may be transmitted from the nonhuman organ donors to the recipient human. Additionally, information regarding these transmissible agents may provide valuable information about the organ donors' cellular receptors that may be important for transplantation success.

Gene therapies have long looked for a good vector that can transport the foreign gene of choice into human cells. The lack of any known disease associated with the virus of the present invention makes the present invention an ideal candidate for gene therapy regimens. Thus, compositions and methods for gene therapy are needed that use a vector capable of carrying a significant amount of foreign DNA that will enter the host organism and not cause disease.

Compositions and methods for vaccination using recombinant live retroviruses are also needed. A live virus, that causes no illness in humans, and that has genes of antigens of choice incorporated into its genome, would provide for an excellent vaccination tool. The retrovirus would reproduce in the human host and expose the immune system to antigens so that an immune response can be initiated.

Targeted attack on reproducing cells is a goal of cancer treatment. What is needed is are compositions and methods for cancer treatment that are specific for dividing cells that do not cause systemic damage to the cancer patient. A virus that could infect and kill dividing cells, without killing other cells of the host would provide a solution for cancer treatment.

Summary of the Invention

The present invention is directed to compositions and methods comprising a novel spumavirus or foamy virus, known as SVFHu-1. The present invention comprises a spumavirus isolate of human origin that has been definitively isolated from a human with no disease. The novel spumavirus of the present invention has been maintained through tissue culture cells where it causes the characteristic vacuolation of the cells that is known for foamy viruses.

The novel spumavirus of the present invention has utility as a reagent for the immunological screening of the human population for the prevalence of such viruses in the population. The novel spumavirus of the present invention can also serve as a vector in gene therapy because the virus appears to cause no disease in humans and is not transmitted to other humans. Additionally, the novel spumavirus of the present invention can be used as a reagent in pathogenicity studies of these and related viruses. Moreover, the sequences of the novel spumavirus of the present invention can be used as probes to detect virus in biological samples. Vectors include, but are not limited to, procaryotic, eucaryotic and viral vectors. The foamy virus of the present invention can also be used as a live recombinant virus vaccine. Additionally, the spumavirus of the present invention can be used as a replicating viral system to kill live dividing cells, either *in vitro* or *in vivo*.

The spumaviruses or foamy viruses are by far the least well characterized of the retroviruses. They have been isolated as agents that cause vacuolation ("foaming") of cells in culture from a number of mammalian species, including monkeys, cattle, cats, and reportedly in humans. Persistent infection with these viruses is not associated with any known disease.

Recent studies using improved diagnostic assays have shown no evidence of foamy virus infection of humans in studies of large populations (approximately 8,000 persons). Given these results, the identification of seroreactivity in three persons occupationally exposed to non-human primates is notable. The PCR identification of viral genome sequences in biologic specimens from all three, and isolation of the virus from one, confirm virus infection in these workers.

The present invention includes the isolation and characterization of a spumavirus, SVFHu-1, that was shown to have been transmitted from non-human primates to humans at some point in the past. The retrovirus of the present invention, like another retrovirus of a more virulent nature, HIV-1 (Human Immunodeficiency Virus) The spumavirus of the present invention does not appear to be readily transmitted from human to human. The spumavirus of the present invention can be used in constructing protocols for diagnosing spumavirus infections and may be used as a vector in gene therapy procedures.

The present invention also includes methods and compositions for detecting spumavirus in biological fluids. The methods and compositions, including kits, can be in any configuration well known to those of ordinary skill in the art. The present invention also includes antibodies specific for the spumavirus and antibodies that inhibit the binding of antibodies specific for the spumavirus. These antibodies can be polyclonal antibodies or monoclonal antibodies. The antibodies specific for the spumavirus can be used in diagnostic kits to detect the presence and quantity of spumavirus in biological fluids or in organs from nonhuman primates for xenotransplantation. Antibodies specific for spumavirus may also be administered to a human or animal to passively immunize the human or animal against spumavirus, thereby reducing infection after accidental exposure to nonhuman primate bodily fluids.

The present invention also includes compositions and methods, including kits, for detecting the presence and quantity of antibodies that bind spumavirus in body fluids. The methods, including kits, can be in any configuration well known to those of ordinary skill in the art. Such kits for detection of spumavirus itself or detection of antibodies to the spumavirus can be used to monitor the blood supply for the presence of spumavirus in the blood supply.

The present invention also includes methods and compositions comprising recombinant live virus vaccines. The virus of the present invention has areas of its genome that make it ideal for the insertion of exogenous genes. The genes can code for any protein for which vaccination or gene therapy is desired. Because SFVHu-1 replicates at a higher level than other known foamy viruses, it is capable of providing a high level of antigen to the host carrying the virus. After administration of SFVHu-1 to the host, the virus would infect the cells, replicate and provide protein antigens to the immune system of the host. A novel aspect of such recombinant live viruses is that SFVHu-1 does not cause disease in the host organism. Additionally, there is no transmission from one host organism to other non-infected host organisms, even by close contact with exchange of bodily fluids. The recombinant live virus vaccines of the present invention are a safe way to provide antigen in a most optimum method to the immune system.

The present invention further includes methods and compositions for the use of replicating viral system to kill live dividing cells in a host or *in vitro*. In *in vitro* uses, SFVHu-1 can be used to detect and kill rapidly dividing cells. Foamy viruses, including SFVHu-1, can infect a wide variety of species of cells and can be used in many *in vitro* cell systems. For example, if the assay of the *in vitro* cell system required the identification of quiescent cells, application of SFVHu-1 to the tissue culture system would result in the selection of the rapidly dividing cells by SFVHu-1. The tissue culture cells would be infected, but because SFVHu-1 has a productive infection and cytopathic effects only in dividing cells, the dividing cells are killed by such dividing cells would be infected by SFVHu-1 and killed by such infection. The remaining non-dividing cells of the culture would remain alive.

In a host, the ability of SFVHu-1 to infect dividing cells provides an excellent treatment for conditions due to the presence of rapidly dividing cells. For example, a person with disease due to rapidly dividing cells, such as cancer or any known angiogenic condition, could be infected with SFVHu-1. Such virus may or may not carry other, exogenous genes for other effects in the host. Because SFVHu-1 does not cause disease in the host and there is no transmission of the virus to contacts with the host, only the person with the disease from rapidly dividing cells will be treated. In addition, only the rapidly dividing cells of that host person will be infected by SFVHu-1, and the rest of the body will remain uninfected. The virus will infect the rapidly dividing cells and kill them. For example, a person with a fast growing tumor would be infected with SFVHu-1 and the cells of the tumor would be destroyed by the virus. The SFVHu-1 can be recombinantly modified to be selective for cellular receptors on the tumor to make the virus even more specifically targeted to just those cells.

Such treatment with SFVHu-1 could be used for any condition in which rapidly dividing cells provide an aspect of the pathology of the condition. One such condition is the presence of uncontrolled angiogenesis within the body. Angiogenesis dependent diseases are well known in the art and are caused in part by the rapid growth of blood vessels.

Accordingly, it is an object of the present invention to provide a composition comprising a novel spumavirus.

It is another object of the present invention to provide a method of detecting a spumavirus.

It is yet another object of the present invention to provide methods and compositions for detecting the presence and amount of spumavirus in a body fluid or organ.

A further object of the present invention is to provide compositions and methods for treating genetic and physiologic disorders using gene therapy techniques comprising the novel spumavirus of the present invention as a vector for nucleic acid sequences and antisense sequences.

Another object of the present invention is to provide compositions and methods useful for manipulating the expression of genes.

Yet another object of the invention is to provide vaccines.

Yet another object of the present invention is to provide compositions and methods for treating viral infections in humans or animals.

Another object of the present invention is to provide compositions and methods that are effective in treating genetic diseases.

Yet another object of the present invention is to provide a method of treating microbial infections in humans or animals.

It is another object of the present invention to provide for treatments of conditions that are caused in part by rapidly dividing cellular growth.

Another object of the present invention is to provide live recombinant virus vaccines.

An object of the present invention is to provide diagnostic tools such as antibodies or antigens for the monitoring of the blood supply or organ and tissue donation for the presence of spumavirus.

These and other features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

Brief Description of the Drawings

Figure 1 shows a transmission electron microscope photomicrograph of viral particles in Cf2Th canine thymocytes.

Figure 2 shows tissue culture AMP-reverse transcriptase activity in canine thymocyte cells (Cf2Th) co-cultured with peripheral blood lymphocytes from an infected case worker. Along the baseline is another

line showing control Cf2Th cells that were co-cultured with normal human peripheral blood lymphocytes, indicating there was no constitutive reverse transcriptase activity in these cultures .

Figure 3 is a Western blot of sera from Case A, Case B and Case C and the sera of spouses of two of the cases. The sera was tested against the whole cell lysate from Cf2Th cells infected with the spumavirus isolate. Whole cell lysate of uninfected Cf2Th were used as a control for seroreactivity towards nonviral proteins. In addition, the sera of Case B provides a view of the history of infection because of the existence of Case B sera obtained in 1967, and in 1978, 1980, and 1981.

Figure 4 is a phylogenetic tree showing the relationships between the sequences of the viruses of the novel spumavirus of the present invention and known spumaviruses.

Figure 5 is a comparison of the nucleotide homology of the sequenced portion of the present invention and other retroviruses.

Detailed Description of the Invention

In response to the identification of simian immunodeficiency virus infection in an occupationally exposed workers, Centers for Disease Control and National Institutes for Health collaborated in an anonymous serosurvey of persons with similar work exposures. Simian immunodeficiency virus seroreactivity was present in 3/427 (0.64%) stored serum samples from these anonymous workers (See CDC. Anonymous survey for simian immunodeficiency virus (SIV) seropositivity in SIV laboratory researchers -- United States, 1992. MMWR *Morb Mort Wkly Rep* 1992; 41: 814-5; Khabbaz RF, et al.,. Brief report: infection of a laboratory worker with simian immunodeficiency virus. *New Eng J Med.* 1994; 330: 172-7). Consequently, a voluntary testing and counseling program was developed that allowed linkage between specific exposures or health outcomes and serostatus of persons occupationally exposed to simian immunodeficiency virus. The workers enrolled in this voluntary linked prospective simian immunodeficiency virus surveillance are also at occupational risk for exposure to other retroviruses common in nonhuman primates (non-human primates).

Therefore, in 1995, the linked surveillance was expanded to include voluntary testing and counseling for exposure to simian spumaviruses (more

commonly called simian foamy viruses, or SFV), simian T-lymphotropic viruses (STLV), and simian type D retroviruses. 1,823 samples from 13 institutions in the United States had been tested for simian immunodeficiency virus; samples from 231 of the participating volunteer workers were also tested for other retroviruses from non-human primates. Three of these 231 workers (1.3%) were determined to be infected with a SFV-like virus by serology and PCR.

An immunofluorescent assay that was developed using cells infected with SFV serotype 3 identified antibodies to a SFV-like virus in recently collected serum specimens from all three workers. The 3 specimens were also western blot positive, showing reactivity to both p70 and p74 gag precursor bands of SFV-3 antigen. Repeat testing of additional sera obtained from these 3 workers at later time points are also positive in both assays. (These workers or cases are herein identified individually as Case A, Case B, and Case C.)

Additional blood samples from these three cases were tested for SFV proviral DNA sequences using polymerase chain reaction (PCR) assays employing primer sets from two regions of the polymerase gene that are conserved among known primate foamy viruses. All three cases were PCR positive in both regions. The PCR products from one region were sequenced. The sequences from each case were distinct from each other but all showed greater than 80% homology to known non-human primate foamy virus sequences. The partial sequences, produced with DNA polymerase PCR primer, of the viral sequence of the present invention is shown below. Seq. ID 1 is a viral DNA sequence isolated from infected Cf2Th cells and Seq. ID 2 is a viral DNA sequence isolated from PBLs from Case A. There is 99.76 % homology between the two sequences. The corresponding RNA sequences and resulting proteins can be deduced from these sequences.

Seq. ID 1

TTACTACAAGGACAATATCCAAAAGGTTTTCCAAAACAATATCAATATGA

ACTTAATGAAGGACAAGTTATAGTAACTCGTCCTAATGGACAAAGAATTA

TTCCTCCAAAATCAGACAGGCCTCAAATTATTTTGCAAGCACATAATATT

GCACATACAGGAAGAGATTCAACCTTTCTTAAGGTCTCTTCCAAGTATTG

GTGGCCAAATCTTAGAAAGGATGTGGTTAAAGTTATCAGACAATGTAAGC

AATGTCTGGTCACAAATGCAGCTACCTTAGCTGCGCCTCCAATACTGAGG
CCTGAAAGACCTGTAAAGCCTTTTGATAAATTTTTTGTTGACTATATTGG
CCCTTTACCCCCTTCTAATGGGTACTTACATGTCCTTGTAGTAGTCGATG
GTATGACTGGATTTGTATGGTTA

Seq. ID 2

TTACTACAAGGACAATATCCAAAAGGTTTTCCAAAACAATATCAATATGA
ACTTAATGAAGGACAAGTTATAGTAACTCGTCCTAATGGACAAAGAATTA
TTCCTCCAAAATCAGACAGGCCTCAAATTATTTTGCAAGCACATAATATT
GCACATACAGGAAGAGATTCAACCTTTCTTAAGGTCTCTTCCAAGTATTG
GTGGCCAAATCTTAGAAAGGATGTGGTTAAAGTTATCAGACAATGTAAGC
AATGTCTGGTCACAAATGCAGCTACCTTAGCTGCGCCTCCAATACTGAGG**
CCTGAAAGACCTGTAAAGCCTTTTGATAAATTTTTTGTTGACTATATTGG
CCCTTTACCCCCTTCTAATAGGTACTTACATGTCCTTGTAGTAGTCGATG
GTATGACTGGATTTGTATGGTTA

The relationship between each of the isolates and other known spumaviruses is shown in Fig. 5 which is a phylogenetic tree showing the percent homology of the nucleotide sequences of these viruses and in Figure 6.

The 5' end of the LTR of SFVHu-1, of 1567 nucleotide bases, has also been sequenced, and is shown as Seq. ID 3.

1 TTCCCAATAA ACATCATCCT GGGTGGACTA GACATCTTAC TAAATTCAAG
51 ATATCTAGAT TCTCCACTCC TGCTGATGTC CAGAAAATTG TGGATGAGCT
101 TCTCCCTAGA GGAGCAAGCA TTGTAATGCC TGATGGAACA AAGTATCCAA
151 GTACCAGAAA AGTGCACTTA GTCAATGAAG GAACCCTTGT AGAATACCAA
201 GCCAAATGTA AGGAGATAGA GGAAAAGTAC GGAGGATGCT TTTCTACAGA
251 TAGTGATGAT GACAGTGATG ATTACTCTGA GGATACTCCA GAAACTGAAA
301 CCACTGATGT GGAATAGAGT ACAGTGTTAA GGATTCACAT AATCTGCCTA

351 GCAACTGCTT ATGCTTAAGA ATGAATCAGT ATATTGTTTA GGAATAAGTT
401 ATAGTTTATA AGAAGTTAAT CCTTAGGGAG TATTTGGTGG AAATGACTGA
451 GTGACATGAA GTTTATTCAC CATACTCTCA ATAGGAGCCA CTAGTTGAGC
501 CTGTGCGTTC AAATCCATGC TCAGCTTAAG TGACTCCCTT TTAGTTTCAC
551 TTTAAGTTAA GTTAGGAATA AGTTCCATAT AATCCTAAGG GAGTATGTGG
601 ACCTTCTTGT TAGGAAATAG TTTAAGATAG TCCACAGCTC CCTTCTTTTT
651 GAGTTCTAGT CTTTGTTAAG TTTGTTGGCT CATACAGATA AAGTGCTCAT
701 TAAACAGGAA ACCGCAACCG GGTAAAGGTT AGCACAGTAA ATTAAGCTAG
751 CAGTTACTCA AGAGCCCGGT AAGCATTCAA GTAGTTCGAA TCCCTTTAAT
801 GCTGACGGAT TGCTCTTTAG TGAGGTGATG TAATCTGTTT TTGCAATCTG
851 AAATGTGTGT TTGCACAGGA AGTTGTACAA GAAAGGGAAT GGCTAAACTT
901 GTTACAGTTC GAACAAACAT TTAGCAATTT CCTTTGCTTT TGGAGTTCGA
951 GCCTTGTACT TATACTTTGA GCATATGTAT TGTAACACCT AAGTATGGAA
1001 AAATCTCCAA GTATGAGTCA CGAGATGCTT GGCTCACTGC GTTGGACGAC
1051 TGGAAAGAAG CTTCAACAGT CGGGACAGCA TCTCGAAGAA GGCCTCCGGA
1101 ATGAAAGAGT GAAAAATGAA GTCTCCTCAT TCAGAGAGCC TTCTTTTAGA
1151 ATTTCAGGCA GAATAGAGTT TCCAATAGAA TAAACTTTTG TATTAGCAGA
1201 TAGATAGGAT ATATAATCTC TGCTTTAGAT TGTACGGGAG CTCACCACTA
1251 CTCGCTGCGT CGAGAGTGTT CGAGTCTCTC CAGGCTTGGT AAGATATAAA
1301 CTTTGGTATT CTCTGTATTC TTATGATCCA ATATTACTCT GCTTATAGAT
1351 TGTAATGGGC AATGGCAATG CTTTATCAAT GAATGATTTT ATGGTGAATT
1401 AAGTTCATAT ATGTTTTAAG AAGTTTAACA ATAAACCGAC TTAATTCGAG
1451 AACCAGATTT ATTAGTATTG TCTCTTTCTA TACTTTAAGT AAAGTGAAAG
1501 GAGTTGTATA TTAGCCTTGC TTATAAGAGC CATCTAGTGG TATAAGTGTG
1551 TACTACACTT ATCTAAA

A 3' internal region of SFVHu-1 has also been sequenced. This sequence includes ORF 1 (Open Reading Frame) and ORF-2, which are overlapping genes, and includes 3' sequence from env and bel genes. This

sequence is identified as Seq. ID 4 and contains 2406 nucleotides. This sequence is analogous to SFV-3 bases 8953 to 11,356.

Seq. ID 4

1 AAGGGGATGT TGAGCAATCC AACATGTGCA TACCCACTTG AATCATCTTA

51 AAACCATGTT ACTAATGAGG AAGATTGACT GGACTTTTAT TAAGAGTGAT

101 TGGATTAAAG AACAACTTCA GAAAACTGAA GATGAAATGA AGATTATTAG

151 AAGAACAGCT AAAAGTTTAG TATATTATGT GACTCAAACA TCATCTTCCA

201 CTACAGCAAC ATCATGGGAA ATTGGAATTT ATTATGAAAT AACTATACCA

251 AAACATATTT ATTTGAATAA TTGGCAAGTT GTTAACATAG GTCATCTGAT

301 TGAGTCAGCT GGTCATTTGA CCTTAATAAG GGTTAAACAT CCTTATGAAG

351 ACTTTAATAA AGAATGCACA TATGAACAAT ATTTACATCT TGAAGACTGC

401 ATATCTCAGG ATTATGTGAT TTGTGACACG GTACAAATAT TGTCACCATG

451 TGGAAACTCA ACAGTAACCA GTGACTGCCC TGTCACTGCT GAAAAGGTAA

501 AGGAACCATA TATTCAAGTG TCAGCTTTAA AAAATGGAAG CTATTTGGTT

551 CTAACCAGTA GAACAGATTG CTCAATACCA GCATATGTTC CCAGCATTGT

601 AACTGTGAAC GAAACAGTTA AGTGTTTTGG GGTTGAGTTT CATAAACCAC

651 TATACTCAGA AAGTAAAGTC AGCTTTGAAC CACAAGTTCC ACATCTGAAA

701 CTACGCTTGC CACATCTGGT TGGGATTATT GCAAGTCTTC AAAATTTGGA

751 AATTGAAGTA ACNAGCACCC AAGAGAGTAT ANAAGATCAG ATTGAAAGAG

801 TTCAATCACA GCTTCTTCGG CTGGACATTC ACGAGGGAGA CTTTCCTGCT

851 TGGATTCAAC AACTTGCTTC TGCAACCAAG GACGTCTGGC CTGCAGCTGC

901 TAAAGCTCTT CAAGGCATAG GTAACTTTTT ATCTAATACT GCCCAGGGAA

951 TATTTGGAAC TGCTGTAAGT ATTCTATCCT ATGCCAAGCC TATTCTTATA

1001 GGAATAGGTG TTATACTTTT GATTGCATTC TTGTTTAAGA TTGTATCATG

1051 GCTTCCTGGG AAGAAGAAAA AGAACTAGGA CATCTGCATC TTCCAGAAGA

1101 CGATCCTCTG CCCAATTTAG ATGTGCTCCT GGGTCTTGAT CATATGGAAT

1151 CCAATGAAGG ACCTGATCAA AATCCAGGAG CTGAAAAGAT CTACATTCAA

1201 CTCCAAGCAG TCCCAGGGGA AGCCTCAGAG AAAACTTACA AATTTGGATA

1251 TGAAGACAAA GAGGCACAAA ATCCTGACTT AAAAATGAGA AATTGGGTTC

1301 CTAACCCCGA CAAAATGAGT AAGTGGGCCT GTGCAAGGCT TATTCTTTGT
1351 GGACTTTATA ATGCAAAAAA GGCTGGAGAA CTCTTGGCTA TGGACTATAA
1401 TGTTCAATGG GAACAATCAA AAGAAGACCC AGGATACTTT GAAGTGGAAT
1451 ATCACTGTAA AATGTGCATG ACTGTTATTC ATGAACCTAT GCCTATCCAA
1501 TATGATGAAA AAACTGGATT ATGGCTAAAA ATGGGTCCCC TTAGGGGAGA
1551 TATAGGATCT GTAGTACATA CTTGTAGAAG GCATTACATG AGATGTTTGT
1601 CTGCCCTTCC TAGCAATGGA GAACCTCTCA AACCTAGAGT CCGGGCTAAT
1651 CCTGTCCGAA GATATCGAGA GAAGCAAGAG TTCGTTGCGA CTAGGCCTAA
1701 ACGCTCCAGA TGGGGTGTGG CCCCTAGCGC AGACTCCCAT ACTTCCAGTG
1751 GTGACGCCAT GGCCCTTATG CCAGGACCAT GCGGCCCCTT CGGTATGGAC
1801 ACTCCTGGTT GCTTACTGGA AGGGATACAA GGATCAGGGC CTGGAACCTC
1851 CGAAATGGCT GTGGCAATGT CAGGAGGACC TTTCTGGGAA GAAGTGTACC
1901 GGGACTCAAT TCCTGGTGCC CCCACTGGGT CTAGTGAAAA TTAGGCTTTA
1951 TCAAAATCTA ACTGTTGTAA ATGTTTGTGG ATCTGTTGAC CCATGGGAAA
2001 ATGAGAATCC CACTAGAGGT CGCAGAGGGC CTATGCATAG ATATGATTGT
2051 AGAATTGCTT GTGATCCAAG CTATTGCTTT AAGGCTATTT GGGAAGGAAA
2101 CTTTTGGGAC AAAAAAAAAA GGATCAGGCA TGCTGGCTAG TTCATCTGAA
2151 AGAAGGACAT AAATTTGGTG CAGATGAGTT ATCTTCTGGG GATCTTAAAA
2201 TATTAGCAGA ATCTAGACCT TATCCATATG GATCTATTGG TCATTGTGCT
2251 ATGCTTCAAT ATGCAGTACA AGTTAAAATG AGAGTTGATA GAGCTCCTTT
2301 GACCTCAAAG GTGAGAGCTA TTAAAGCTTT GCACTATCAT CGCTGGAATA
2351 TTTGTCAGCT GGAAAATCCT GGCATAGGAG AAGGATTCAG TCCCTCTGGT
2401 AATACACA

Seq. IDs 1-4 can be used for all the molecular biological techniques known to those skilled in the art. Such uses include, but are not limited to, generation of probes and vectors containing the sequences, antisense sequences derived from such sequences, and proteins synthesized using the sequences. RNA and other nucleic acid derivatives are contemplated by the present invention.

The 5' sequenced region of SFVHu-1, shown in Seq ID 3, comprises the LTR (Long Terminal Repeat). In foamy viruses, the LTR aids in the replication of the virus. The LTR is transactivated by a virus-specific protein, and unlike related retrovirus, HIV (Human Immunodeficiency Virus), no human cellular transcription factors activate the virus. LTRs in retroviruses like HIV have conserved consensus sequences for cellular transcription factors.

According to sequence homology, SFVHu-1 Seq ID 3 LTR are stable. There has not been significant change in the sequence even after long passage in a human host. For gene therapy uses, this stability is very important. It also appears that the internal promoter, found in the 3' sequence, Seq ID 4, is also conserved. Thus, the transcriptionally important regions of SFVHu-1 are stable. This indicates that the virus is not acquiring human sequences that would cause it to possibly become virulent or at least cause disease in humans due to introduced mutations. SFVHu-1, because of this stability, is an excellent vector, vaccine or gene therapy agent for humans. This stability is surprising is light of the high instability of the LTR of the virus known as HFV, Human Foamy Virus. HFV was derived from a nasocarcinoma and is now believed not to be a human foamy virus, but a chimpanzee virus. The HFV LTR is unstable and has lots of deletions, thus making it an undesirable vector.

The foamy viruses are unique in that at the 3' end of the env gene there is an internal promoter, IP. ORF 1 codes for a transactivator protein, TAF. TAF activates IP. Once the virus infects the cell, a little TAF is made, this TAF activates the internal promoter IP, which then causes the virus to make lots of TAF. Once sufficient quantity of TAF is made, the TAF functions to initiate the promoter found in the 5' LTR.

ORF 2 has presently unknown function, though it is theorized that it is necessary for replication of the virus *in vivo*. Without all of ORF 2 present, the virus will replicate *in vitro*, but the existing paradigm, prior to the present invention, was that ORF 2 was required for *in vivo* replication. ORF 2 is a putative site for gene insertion. Surprisingly, it has been found in Seq. ID 4, that ORF 2 of SFVHu-1 has multiple stop codons that prevent its translation. SFVHu-1 has a 5 base insertion and a point mutation that prevent accurate translation of ORF 2. According to the existing theory for foamy virus replication *in vitro* discussed above, these mutation should

prevent replication of SFVHu-1 in humans. Surprisingly, the inventors have found that SFVHu-1 has a high rate of replication in the human host. The virus is found in the peripheral blood lymphocytes (PBL) of the host and is cultured from such cells in tissue culture systems. Reverse transcriptase activity has been found in the PBLs and plasma of the infected host. Viral RNA of SFVHu-1 has been shown by viral RT-PCR in both PBLs and plasma of the infected host. No other foamy virus has shown this activity. The literature has reported that there has been no identification of foamy viral replication in humans, until now, with the present invention, no such replication has been shown.

Virus isolation was attempted by co-culturing the PBLs (peripheral blood lymphocytes) of Case A with Cf2Th canine thymocytes, a cell line known to be permissive for spumavirus infection. See Mergia A, et al., "Cell tropism of simian foamy virus type 1 (SFV-1)," J. Med. Primatol. 1996:25:2-7. Reverse transcriptase activity was detected in co-cultures from the cells exposed to Case A PBLs but not from controls. Transfer of supernatant from the above cells exposed to Case A's PBLs passed this reverse transcriptase activity to uninfected cells, which subsequently showed cytopathic effect (CPE). This finding indicated that the infectious agent in Case A's PBLs was transmitted to tissue culture cells which were used to transfer the infectious agent into other tissue culture cells. Additionally, this indicated that the infectious agent reproduced in the Cf2Th canine thymocytes. DNA-PCR of infected cells was found to be positive for a SFV-like virus. Infected cells showed strong reactivity with all 3 cases' sera by both immunofluorescent assay and western blot and no reactivity with normal sera controls. By electron microscopy, infected Cf2Th cells, derived from cell free supernatants from cells infected by exposure to infected PBLs, showed a morphology characteristic of foamy virus infection (See Figure 1).

The present invention is directed to compositions and methods comprising a new spumavirus, SFVHu-1. The virus was isolated from humans who had worked with nonhuman primates. The new spumavirus, or foamy virus, does not appear to cause any disease in the human hosts. The new virus of the present invention may be an excellent vector for gene therapy and for vaccination purposes. Additionally, the antibodies or other detection methods for detecting the new virus may be important in detecting

the presence of this and related viruses for xenotransplantation. In addition, the novel spumavirus of the present invention can be used as a reagent in pathogenicity studies of these and related viruses. Moreover, the sequences of the novel spumavirus of the present invention can be used as probes to detect virus in biological samples. Vectors include but are not limited to procaryotic, eucaryotic and viral vectors.

Many new and potentially useful technologies are being developed which use viral vectors and may form the basis of future medical cures and therapies. Examples of such technologies include, but are not limited to, gene replacement, antisense gene therapy, *in situ* drug delivery, treatment of cancer or infectious agents, and vaccine therapy. However, to be successful, these technologies require an effective means for the delivery of the genetic information across cellular membranes.

The recent advent of technology, and advances in the understanding of the structure and function of many genes makes it possible to selectively turn off or modify the activity of a given gene. Alteration of gene activity can be accomplished many ways. For example, oligonucleotides that are complementary to certain gene messages or viral sequences, known as "antisense" compounds, have been shown to have an inhibitory effect against viruses. By creating an antisense compound that hybridizes with the targeted RNA message of cells or viruses the translation of the message into protein can be interrupted or prevented. In this fashion gene activity can be modulated.

The ability to deactivate specific genes provides great therapeutic benefits. For example, it is theoretically possible to fight viral diseases with antisense molecules that seek out and destroy viral gene products. In tissue culture, antisense oligonucleotides have inhibited infections by herpes-viruses, influenza viruses and the human immunodeficiency virus that causes AIDS. It may also be possible to target antisense oligonucleotides against mutated oncogenes. Antisense technology also holds the potential for regulating growth and development. However, in order for the gene therapy to work, antisense sequences must be delivered across cellular plasma membranes to the cytosol.

Gene activity is also modified using sense DNA in a technique known as gene therapy. Defective genes are replaced or supplemented by the administration of "good" or normal genes that are not subject to the

defect. Instead of being defective, the gene have been deleted, thus replacement therapy would provide a copy of the gene for use by the cell. The administered normal genes can either insert into a chromosome or may be present as extracellular DNA and can be used to produce normal RNA, leading to production of the normal gene product. In this fashion gene defects and deficiencies in the production of a gene product may be corrected. Still further gene therapy has the potential to augment the normal genetic complement of a cell. For example, it has been proposed that one way to combat HIV is to introduce into an infected person's T cells a gene that makes the cells resistant to HIV infection. This form of gene therapy is sometimes called "intracellular immunization." Genetic material such as a polynucleotide sequence may be administered to a mammal in a viral vector to elicit an immune response against the gene product of the administered nucleic acid sequence. Such gene vaccines elicit an immune response in the following manner. First, the viral vector containing the nucleic acid sequence is administered to a human or animal. Next, the administered sequence is expressed to form a gene product within the human or animal. The gene product inside the human or animal is recognized as foreign material and the immune system of the human or animal mounts an immunological response against the gene product. The virus of the present invention may be used as a viral vector to provide the foreign nucleic acid sequences to the intracellular metabolic processes.

Additionally, gene therapy may be used as a method of delivering drugs *in vivo*. For example, if genes that code for therapeutic compounds can be delivered to endothelial cells, the gene products would have facilitated access to the blood stream. Additionally, cells could be infected with a retroviral vector such as the present invention carrying nucleic acid sequences coding for pharmaceutical agents that prevent infection from occurring in the retrovirally infected cells.

The novel spumavirus of the present invention can also be used a safe and effective vaccine agent. Genetic sequences for immunogenic proteins from a variety of infectious agents can be incorporated into the foamy virus RNA. Once inside a cell, the gene product is expressed and releases the immunizing peptide to the body's immune system. In another method, the virus of the present invention can be used to immunize the body against cell markers found on cancer or tumor cells. The genetic sequence

of the cancer cell marker is incorporated into the foamy virus RNA and after infection with the virus, the expressed gene product stimulates the immune system. The patient's immune system is used to remove the cancerous cells, obviating the need for chemotherapeutic methods.

The antibodies of the present invention can be used to detect the presence of the virus or viral particles of the present invention. These antibodies can be used in diagnostic or screening kits to assess the present of the virus. Additionally, the antibodies can be used to screen organs from nonhuman primates that may be used in humans. Detection of the presence of a virus that is transmitted from nonhuman primates to humans would be crucial in providing virus-free organs for transplantation.

The virus of the present invention can be used for the treatment of conditions due to the presence of rapidly dividing cells. In a host, the ability of SFVHu-1 to productively infect dividing cells provides an excellent treatment for conditions due to the presence of rapidly dividing cells. For example, a person with disease due to rapidly dividing cells, including but limited to cancer or any known angiogenic condition, could be infected with SFVHu-1. Such virus may or may not carry other, exogenous genes for other effects in the host. Because SFVHu-1 does not cause disease in the host and there is no transmission of the virus to contacts with the host, only the person with the condition due to rapidly dividing cells will be treated. In addition, only the rapidly dividing cells of that host person will be productively infected by SFVHu-1. Other cells in the body may be infected but will not be killed because the infection in nondividing cells is not productive. The virus will productively infect the rapidly dividing cells and kill them. For example, a person with a fast growing tumor would be infected with SFVHu-1 and the cells of the tumor would be destroyed by the virus. Additionally, the virus may be given to a person prior to the person developing a condition caused by dividing cells, and when the cells begin dividing, the virus would then undergo a productive infection and kill the cells. This therapy may halt or inhibit such conditions as leukemia or angiogenesis dependent diseases such as macular degeneration.

Such treatment with SFVHu-1 could be used for any condition in which rapidly dividing cells provide an aspect of the pathology of the condition. One such condition is the presence of uncontrolled angiogenesis within the body. Angiogenesis dependent diseases are well known in the art

and are caused in part by the rapid growth of blood vessels. Another such condition is cancer or tumor growth. Cancer or tumors include both solid tumors and other types. Infection with the virus of the present invention, which causes no disease and does not effect the host systemically, is an improvement over currently known treatments that involved systemically administered agents. Such chemotherapeutic agents kill rapidly dividing cells but also cause trauma to the entire person. The dosages of such chemotherapeutic agents must be titered between killing the cancer and killing the patient.

In contrast, treatments of cancer with the present invention are not as harmful to the patient. The virus can either be administered systemically or injected *in situ* into the tumor. The virus will only replicate in rapidly dividing cells and will not effect cells that are not dividing. The infected cells are killed and tumor growth is stopped. The virus may be administered in one treatment or in a series of treatments.

The SFVHu-1 of the present invention can be recombinantly modified to be selective for cellular receptors on the tumor to make the virus even more specifically targeted to just those cells. Additionally, the virus may have altered promoter regions that can be selectively activated to cause a productive infection. The combination of different levels of control of the virus, both natural and recombinantly produced, are contemplated in the present invention. A virus could be made specific for attachment to only certain types of cellular receptors, for those cells that are dividing, and will only undergo replication if another exogenous promoter factor is present. Viral infection by two or more individually defective viruses, that require factors or promoters supplied by other foamy viruses or any type of virus, could provide for many levels of control of infection or treatment of specific conditions.

The virus may be administered to the host, for cancer treatment, gene therapy or vaccination by any methods known to those skilled in the art. Such methods include but are not limited to injection, inhalation, ingestion, topical administration and implantation. The virus may be killed or live, depending on the treatment considered.

The inventors of the present invention believe that the viruses of the present invention, comprising the isolates from Cases A, B, and C, and particularly Case A, are the first definitive isolation of an SFV-3-like

spumavirus from persons exposed to nonhuman primates. The virus does not appear to cause disease and does not appear not transmitted to close household contacts or sexual contacts. This belief is supported by the epidemiology data, the PCR and sequencing data and the serology data.

The isolate from Case A, SFVHu-1, was deposited with the ATCC under the Budapest Treaty on February 5, 1998, and was assigned ATCC no. ________.

The present invention is further described by the examples which follow. Such examples, however, are not to be construed as limiting in any way either the spirit or scope of the present invention. In the examples, all parts are parts by weight unless stated otherwise.

Example 1

Case A

Case A has intermittently been employed as a caretaker for non-human primates for twenty years between 1961 and 1997. Case A recalled multiple minor injuries and mucocutaneous exposures to non-human primate blood, body fluids, or fresh tissue. In addition, Case A was twice bitten by African green monkeys in the 1960s or early 70s. These injuries were severe enough to require 7-10 stitches each. Case A is single and in good health. No sera collected from Case A prior to 1995 or from sexual partners are currently available for testing. Retrospective analysis of sera archived from Case A in 1995 showed the sera to have antibodies to SFV. (See Figure 3, lane 2).

The western blot of Figure 3 shows whole cell lysate from Cf2Th cells infected with the spumavirus of the present invention tested in each individual lane with different antisera. In Figure 3, particular viral proteins that show infection are the proteins with molecular weight of approximately 70-80 Daltons (p70 gag protein) and the proteins at approximately 130-140 Daltons (an envelope protein). The western blot of Figure 3 shows whole cell lysate from Cf2Th cells infected with the spumavirus of the present invention. These proteins are not detectable in the western blot of Figure 3 by normal sera, (lane 1) but are detectable by antisera from Case A.

Example 2

Case B

Case B is a research scientist employed for three decades working with biologic specimens from non-human primates. Case B rarely reported injuries involving non-human primate blood, body fluids, or unfixed tissue, but did report an injury in 1970 when an unused needle was stuck through a glove that was potentially contaminated with baboon body fluids; and a 1972 cut inflicted by a broken capillary tube containing chimpanzee blood. Case B is in good health. Case B has been in a monogamous sexual relationship without use of barrier contraceptives or spermicides for over 20 years. Case B's spouse is negative for SFV-like infection by both serologic and PCR testing. Analysis of two serum specimens from Case B archived serially in 1967 were negative; sera archived in 1978 and subsequently were consistently seropositive. See Figure 3, lanes 3 and 4 are the 1967 sera, lane 5 is sera from 1978, lane 6 is sera from 1980, lane 7 is sera from 1981. The sera of Case B's spouse is shown in lane 10.

Example 3

Case C

Case C is an animal care supervisor who has worked with non-human primates for more than 3 decades. Case C recalls multiple minor injuries and mucocutaneous exposures to non-human primate blood, body fluids, or unfixed tissues. Case C reported a severe baboon bite around 1980 that required multiple stitches of an arm and hand. Case C is in good health except for type II diabetes mellitus. Case C has been in a monogamous sexual relationship for nearly three decades, during which barrier methods of contraception have not been employed and spermicides were used for no more than a 6 month period. Case C's spouse is negative for SFV-like infection by both serologic and PCR testing. Retrospective analysis of sera archived from Case C in 1988 showed the sera to have antibodies to SFV. See Figure 3, lane 8 is Case C's sera from 1988, and lane 11 is sera from the spouse of Case C.

Example 4

Western Blot Analysis

The sera from the three cases was analyzed by western blot analysis against whole cell lysates from Cf2Th cells infected by cell free supernatants from Cf2Th cells infected by a Case's PBLs. As shown in Figure 3, Case A, Case B and Case C all show the characteristic gag proteins associated with the spumavirus. It is interesting to note that in Case B, Case B converted from negative to positive between 1967 and 1978. In addition, spouses of two of the Cases were negative.

Example 5

Simian Foamy Virus Isolation

Peripheral blood lymphocytes (PBLs) were isolated from Cases A, B and C and were cultured with IL-2 for 48 hours, in RPI media with 10% fetal Calf serum, and penn-strep antibiotics. After 48 hours, the PBLs were added to the Cf2Th cells and co-cultured for 2-4 weeks. The cells were in DMEM supplemented with 2% nonessential amino acids, 20% fetal calf serum, and pen-strep antibiotics. 1 mL supernatants were collected from the cell cultures every 3 to 4 days and tested for amp-reverse transcriptase. Procedures for PBL treatment, culturing of Cf2Th cells and amp reverse transcriptase activity were procedures known to those in the art. For example, see Heneine, W., et al. "Detection of reverse transcriptase by a highly sensitive assay in sera from persons infected with HIV-1." (1995). J. infectious Diseases, 171:1201-6.

Example 6

Because of the positive amp-reverse transcriptase activity from cells from Case A, peripheral blood lymphocytes from Case A were cultured with IL-2 for 48 hours prior to addition to canine thymocytes (Cf2Th), human lung fibroblasts, and normal human peripheral blood lymphocytes. Supernatants were collected every 3 to 4 days and tested for amp-reverse transcriptase activity. Each time the 1 mL sample of supernatant was taken for amp-reverse transcriptase activity, a 5 mL sample of supernatant was taken and frozen at -80 ° C in order to preserve a sample of the virus producing the amp-reverse transcriptase activity.

At day 5, amp-reverse transcriptase testing showed a slightly positive signal in the canine thymocyte culture. The amp-reverse transcriptase activity increased over time. (See Figure 2).

The activity in control Cf2Th cells that were treated as above, except for exposure to normal PBLs instead of infected PBLs, was shown by the lower line that overlaps the baseline. There was no amp-reverse transcriptase activity inherently in these Cf2Th cells, providing evidence that there was no contamination by a retrovirus or spumavirus by the tissue culture cells.

Example 7

At the peak of amp-reverse transcriptase activity as described in Example 5, cell-free supernatants were transferred to fresh Cf2Th growing at 2 x 10^5 cells/mL. At day 4 in the new culture, cytopathic effects and syncytia was observed. Transmission electron microscopy showed viral particles in and around the cells (See Figure 1). Viral particles were isolated from these cultures and were stored at the Centers for Disease Control and will be deposited at the ATCC.

The Cf2Th cells were obtained from the in-house cell culture facility of the Centers for Disease Control, but these cells can also be obtained from the American Type Culture Collection (Rockville, MD). See Mergia et al., et al., "Cell tropism of the simian foamy virus type 1 (SFV-1)," J. Med. Primatol. 1996:25:2-7, for use of these cells.

Having thus described the invention, numerous changes and modifications thereof will be readily apparent to those having ordinary skill in the art, without departing from the spirit or scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Centers for Disease Control
(B) STREET: 1600 Clifton Road
(C) CITY: Atlanta
(D) STATE: Georgia
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 30033
(G) TELEPHONE: 404-639-1024
(H) TELEFAX: 404-639-1174

(ii) TITLE OF INVENTION: New Retrovirus Isolated from Humans

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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AAGGTCTCTT CCAAGTATTG GTGGCCAAAT CTTAGAAAGG ATGTGGTTAA AGTTATCAGA    240

CAATGTAAGC AATGTCTGGT CACAAATGCA GCTACCTTAG CTGCGCCTCC AATACTGAGG    300

CCTGAAAGAC CTGTAAAGCC TTTTGATAAA TTTTTTGTTG ACTATATTGG CCCTTTACCC    360

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TTA                                                                  423
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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CAATGTAAGC AATGTCTGGT CACAAATGCA GCTACCTTAG CTGCGCCTCC AATACTGAGG    300

CCTGAAAGAC CTGTAAAGCC TTTTGATAAA TTTTTTGTTG ACTATATTGG CCCTTTACCC    360

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1567 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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TTTCTACAGA TAGTGATGAT GACAGTGATG ATTACTCTGA GGATACTCCA GAAACTGAAA 300

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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AAGTAAAGTC AGCTTTGAAC CACAAGTTCC ACATCTGAAA CTACGCTTGC CACATCTGGT 720

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CTTTTGGGAC AAAAAAAAAA GGATCAGGCA TGCTGGCTAG TTCATCTGAA AGAAGGACAT 2160

AAATTTGGTG CAGATGAGTT ATCTTCTGGG GATCTTAAAA TATTAGCAGA ATCTAGACCT 2220

TATCCATATG GATCTATTGG TCATTGTGCT ATGCTTCAAT ATGCAGTACA AGTTAAAATG 2280

AGAGTTGATA GAGCTCCTTT GACCTCAAAG GTGAGAGCTA TTAAAGCTTT GCACTATCAT 2340

CGCTGGAATA TTTGTCAGCT GGAAAATCCT GGCATAGGAG AAGGATTCAG TCCCTCTGGT 2400

AATACACA 2408

CLAIMS

What is claimed:

1. An isolated spumavirus cross-reactive with SFV-3 antibodies.

2. The spumavirus of Claim 1, wherein the spumavirus is isolated from a human.

3. The spumavirus of Claim 1, wherein the spumavirus is capable of infecting humans.

4. The spumavirus of Claim 1, wherein the spumavirus is not readily transmitted from human to human.

5. The spumavirus of Claim 1, the spumavirus having ATCC Deposit Nos. ____________.

6. A vector comprising a sequence from an isolated spumavirus cross-reactive with SFV-3 antibodies.

7. The spumavirus of Claim 6, wherein the spumavirus is isolated from a human.

8. The spumavirus of Claim 6, wherein the spumavirus is capable of infecting humans.

9. The spumavirus of Claim 6, wherein the spumavirus is not readily transmitted from human to human.

10. The spumavirus of Claim 6 having ATCC Deposit Nos. ____________.

11. The vector of Claim 6 wherein the vector is a procaryotic vector.

12. The vector of Claim 6 wherein the vector is a eucaryotic vector.

13. The vector of Claim 6 wherein the vector is a viral vector.

14. The vector of Claim 6 wherein the sequence is derived from Seq ID. 1.

15. The vector of Claim 6 wherein the sequence is derived from Seq ID. 2.

16. The vector of Claim 6 wherein the sequence is derived from Seq ID. 3.

17. The vector of Claim 6 wherein the sequence is derived from Seq ID. 4.

18. The vector of Claim 6 wherein the vector is a probe.

19. A method of treating conditions in humans caused by rapidly dividing cells, comprising administration of a spumavirus isolated from humans.

20. A method of gene therapy, comprising administration of a spumavirus containing novel genes to a human.

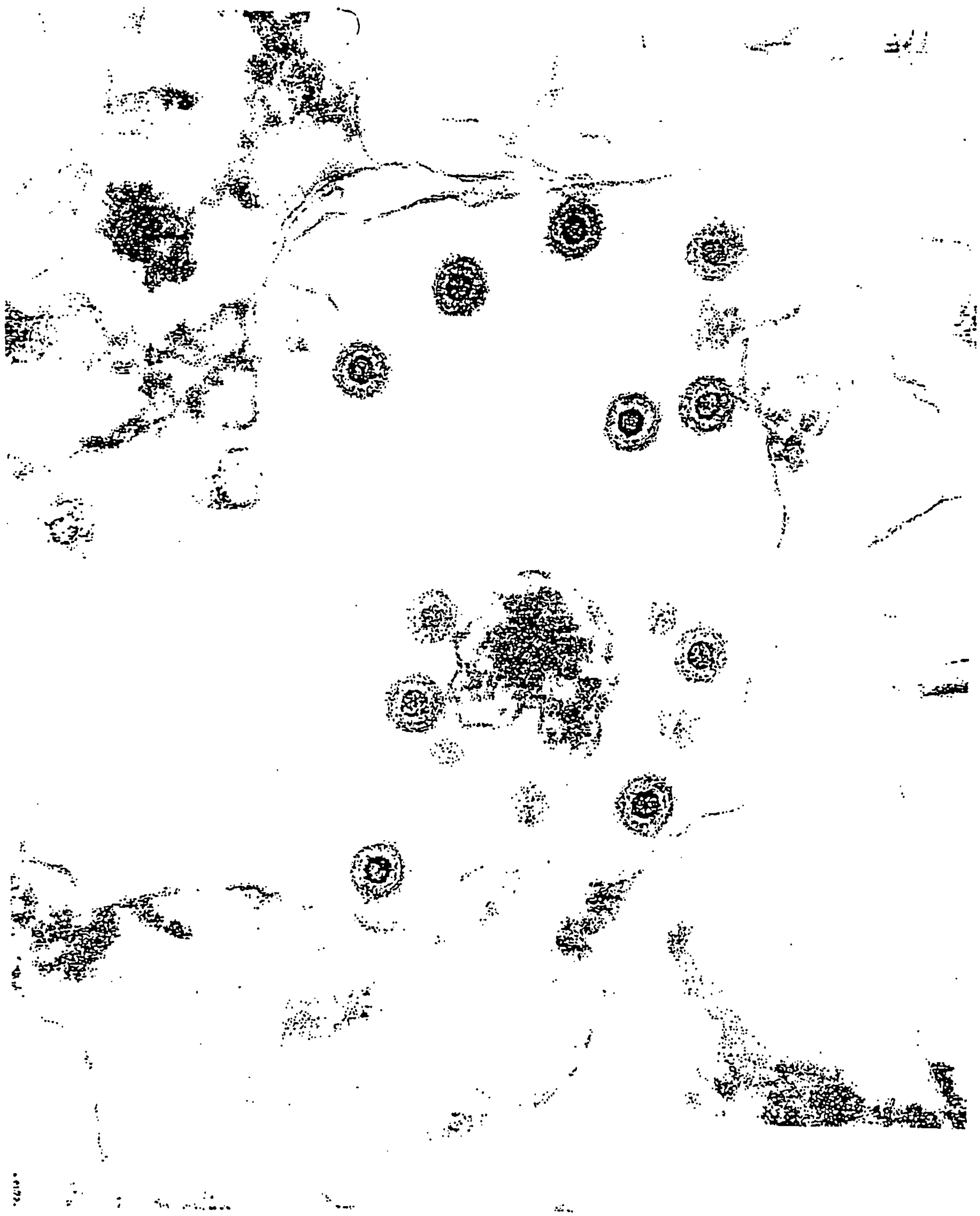


FIG. 1

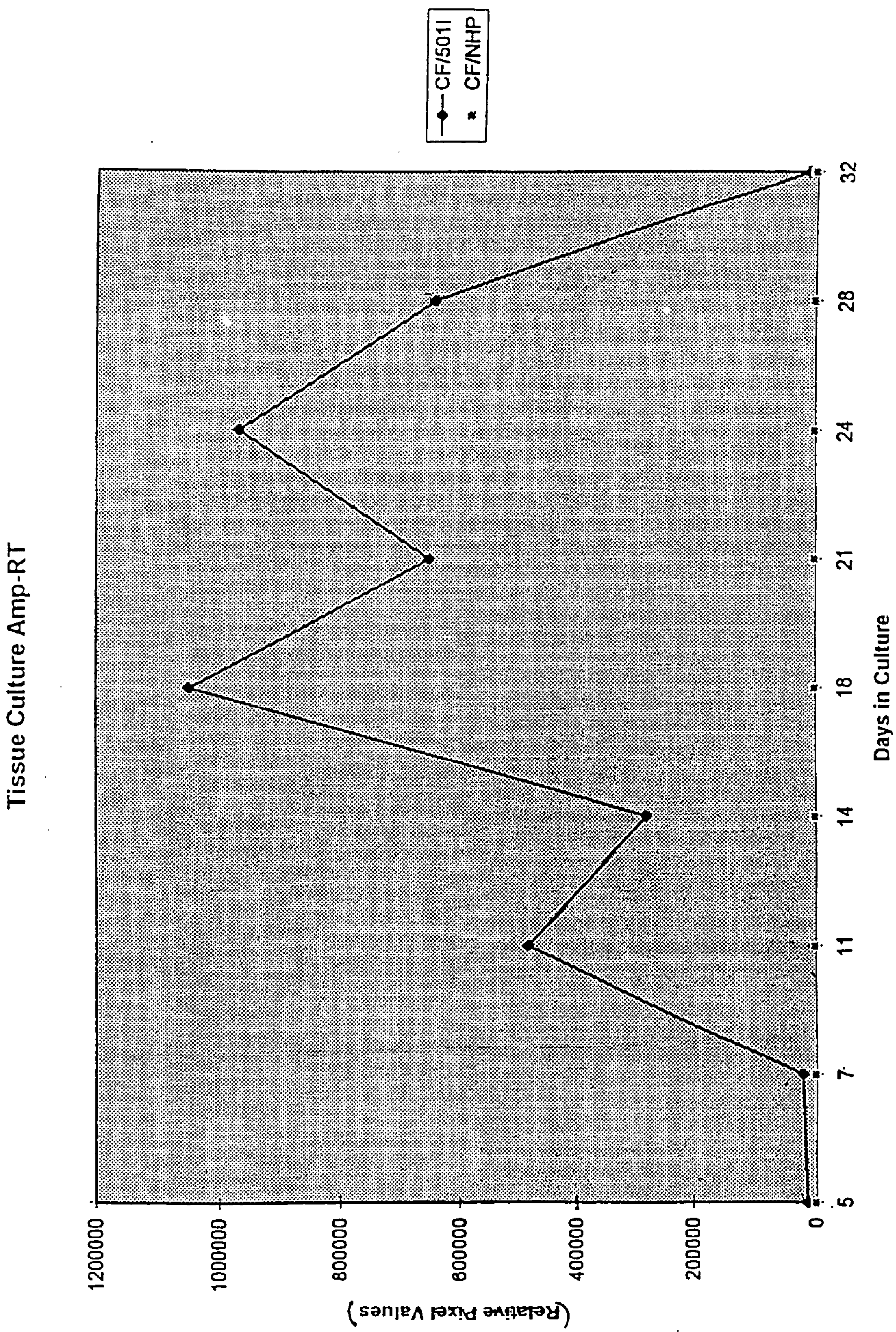
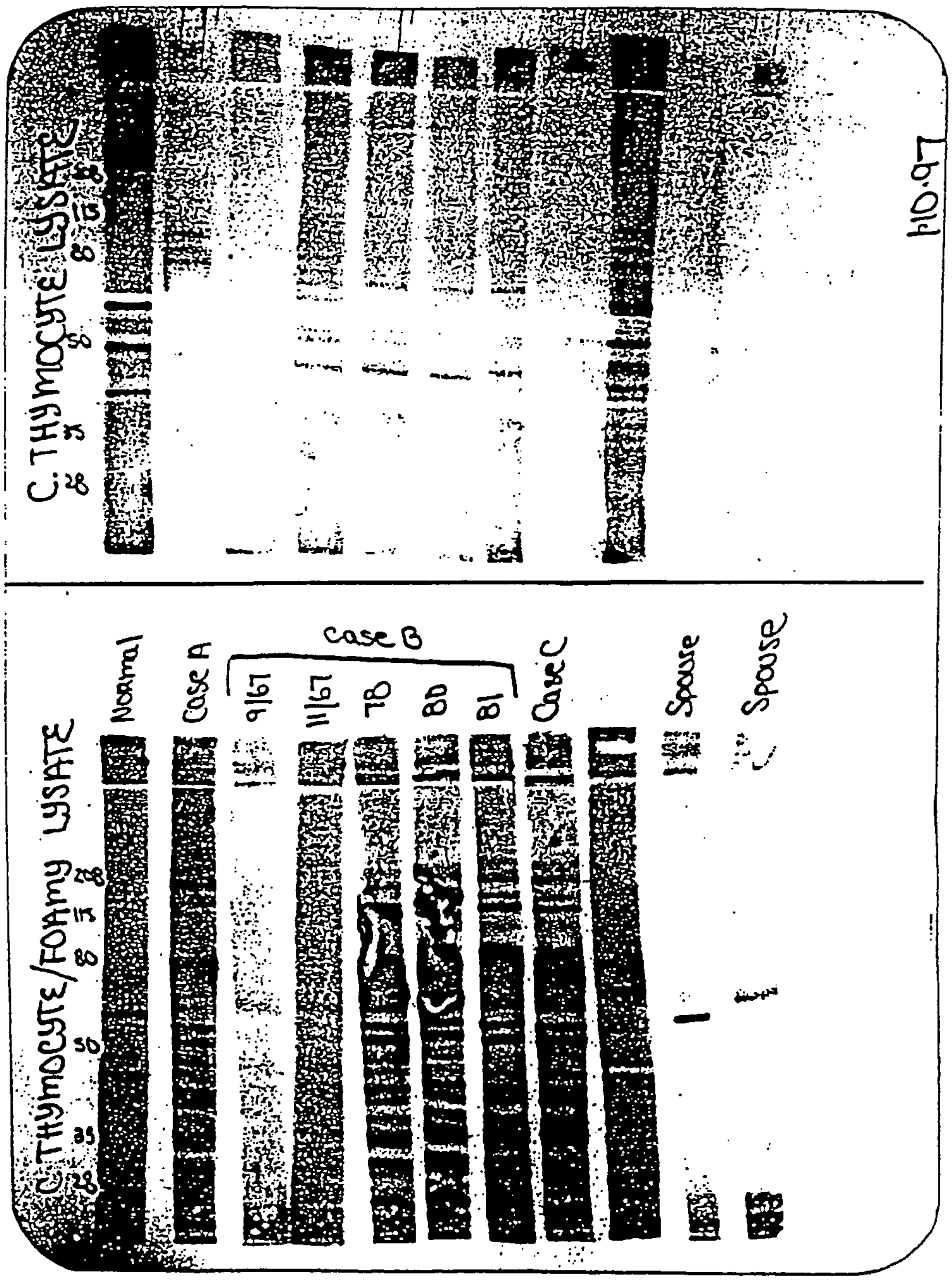

Tissue Culture Amp-RT
CF/501I
CF/NHP
(Relative Pixel Values)
1200000
1000000
800000
600000
400000
200000
0
Days in Culture
5
7
11
14
18
21
24
28
32

FIG. 2

FIG. 3


C. THYMOCYTE/FOAMY LYSATE
Normal
Case A
case B
9/67
11/67
78
80
81
Case C
Spouse
Spouse
C. THYMOCYTE LYSATE

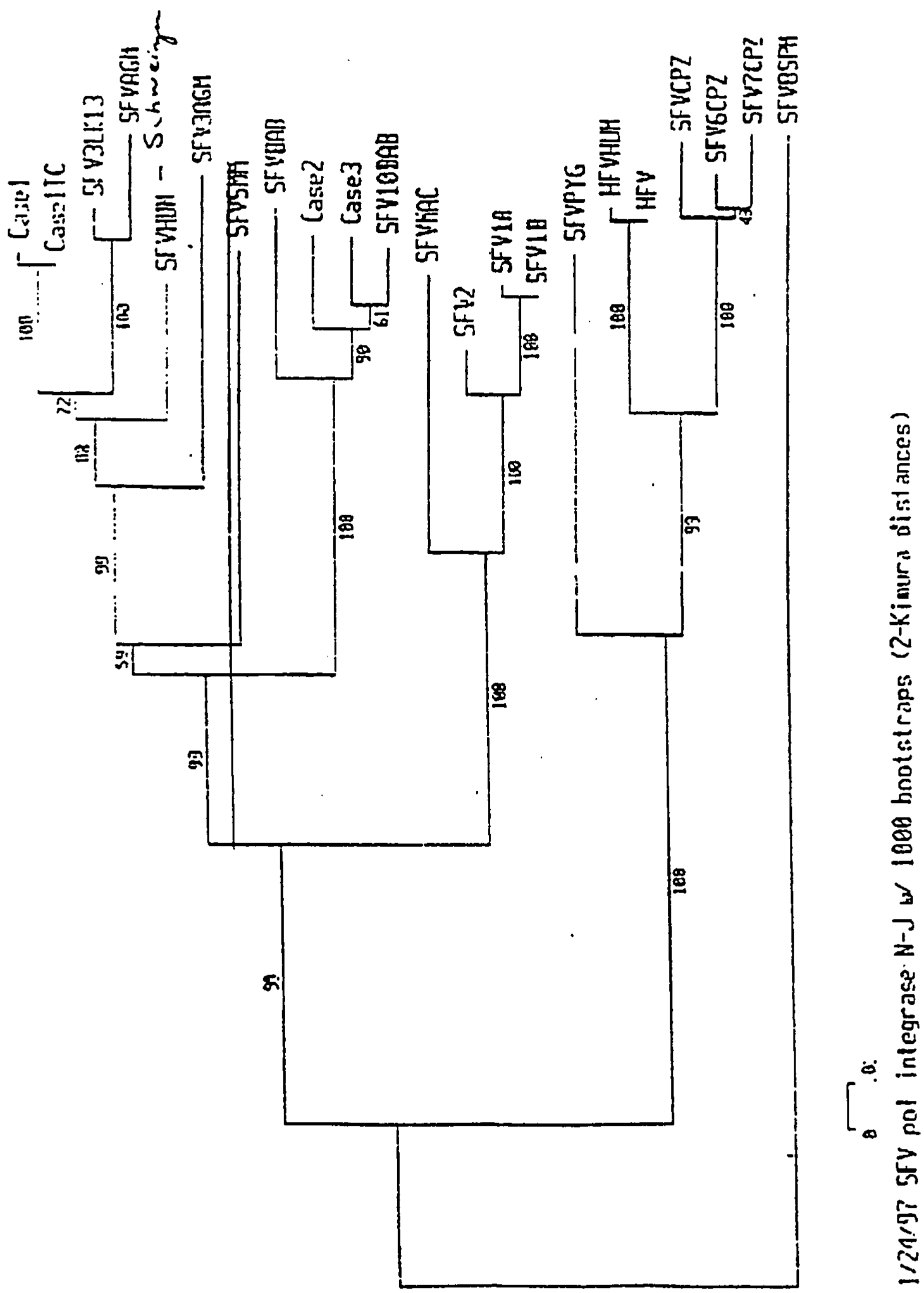

Case1
Case1TC
SFV3LK13
SFVAGM
SFVHUM
SFV3AGM
SFV5MM
SFVBAB
Case2
Case3
SFV10BAB
SFVMAC
SFV2
SFV1A
SFV1B
SFVPYG
HFVHUM
HFV
SFVCPZ
SFV6CPZ
SFV7CPZ
SFV8SPM
100
100
72
100
99
59
99
99
100
90
61
100
100
100
100
100
99
100
100
43
1/24/97 SFV pol integrase N-J w/ 1000 bootstraps (2-Kimura distances)

FIG. 4

Simian Foamy Virus Percent Nucleotide Identity

	Case1	Case2	Case3	SFV 3 AGM	SFV BAB	SFV MAC	HFV	SFV CPZ	SFV PYG	SFV 8 SPM
Case1	-	82.6	82.1	87.5	82.4	77.4	68.7	66.6	67.2	66.4
Case2	-	-	95.5	81.7	92.7	76.2	68.3	66.4	68.9	62.3
Case3	-	-	-	82.1	93.9	76.9	67.5	66.5	69.3	62.3

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/02598

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N7/00 C12N15/86 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAHN H. ET AL.: "Reactivity of primate sera to foamy virus Gag and Bet proteins" JOURNAL OF GENERAL VIROLOGY., vol. 75, October 1994, READING GB, pages 2635-2644, XP002066887 see abstract see page 2638 - page 2639 see figure 3 ---	1-4
Y	DE 43 18 387 A (BAYER AG) 8 December 1994 see the whole document ---	1-4, 6-13, 18-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 1998	09. 07. 1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Panzica, G

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/02598

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHWEIZER M. ET AL.: "Phylogenetic analysis of primate foamy virus by comparison of pol sequences" VIROLOGY, vol. 207, 1995, ORLANDO US, pages 577-582, XP002066888 see the whole document	1-4, 6-13, 18-20
A	RENNE R. ET AL.: "Genomic organisation and expression of simian foamy virus type 3 (SFV-3)" VIROLOGY, vol. 186, 1992, ORLANDO US, pages 597-608, XP002066889	1-4
Y		14-17
A	HIRATA R. ET AL.: "Transduction of hematopoietic cells by foamy virus vectors" BLOOD, vol. 88, no. 9, 1 November 1996, BROOKLINE, US, pages 3654-3661, XP002066890	6-13,18
Y		14-17
A	RUSSELL D.W., MILLER A.D.: "Foamy virus vectors" JOURNAL OF VIROLOGY, vol. 70, no. 1, January 1996, BALTIMORE, US, pages 217-222, XP002066891	6-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/02598

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19 and 20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. .ıal Application No
PCT/US 98/02598

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4318387 A	08-12-1994	EP 0632129 A	04-01-1995
		JP 6343477 A	20-12-1994
		US 5646032 A	08-07-1997